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Review Article

T Cell Receptor Mediated Signalling: An Interactive Pathway for Cytokine Production in Tuberculosis

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Received: April 10, 2019; **Published:** August 16, 2019

Abstract

TB is a global emergency and remains major bacterial causes of mortality. Globally in there were an estimated 10.0 million new (incident) TB cases in 2017 [1]. Although predominantly TB is a disease of lung parenchyma i.e. (pulmonary TB), but it can involve a number of extrapulmonary sites. The resurgence of TB worldwide has intensified research efforts directed at examining the host defence and pathogenic mechanisms operative in *Mycobacterium tuberculosis* (*M. tuberculosis*) infection. *M. tuberculosis* is a classic example of a pathogen for which the protective response relies on cell mediated immunity. This is primarily because the organism lives within cells thus T cell effector mechanisms, rather than antibody are required to control or eliminate bacteria. The detection and interpretation of signals from the environment is an indispensable feature of all cells, including those of the immune system. Although there are an enormous number of different signal-transduction pathways, some common themes are typical of these crucial integrative processes.

Keywords: *T Cell Receptor; Cytokine; Tuberculosis; Mycobacterium tuberculosis*

Introduction

Signal transduction begins with the interaction between a signal and its receptor. Signals that cannot penetrate the cell membrane bind to receptors on the surface of the cell membrane. This group includes water-soluble signalling molecules and membrane-bound ligands (MHC-peptide complexes, for example). Signals are often transduced through G proteins, membrane-linked macromolecules which act as molecular switches. Signal reception often leads to the generation within the cell of a "second messenger," a molecule or ion that can diffuse to other sites in the cell and evoke changes. Examples are cyclic nucleotides (cAMP, cGMP), calcium ion (Ca^{2+}) and membrane phospholipid derivatives such as diacylglycerol (DAG) and inositol triphosphate (IP3). Protein kinases (PKC) and protein phosphatases are activated or inhibited. Signals are amplified by enzyme cascades. Each enzyme in the cascade catalyzes the activation of many copies of the next enzyme in the sequence, greatly amplifying the signal at each step and offering many opportunities to modulate the intensity of a signal along the way. When the default setting for signal-transduction pathways is off in the absence of an appropriately presented signal, transmission through the pathway does not take place.

Initiation of multiple signalling pathways by TCR Engagement

The central event in the generation of both humoral and cell mediated immune responses is the activation and clonal expansion of T cells. The key element in the initiation of T-cell activation is the recognition by the T cell receptor (TCR) of MHC-peptide complexes on antigen-presenting cells. Communication of TCR αβ engagement by peptide-MHC to the intracellular signalling machinery occurs via the TCR-associated CD3 chains, which are arranged into three dimers: γε, δε, and ζζ. Each CD3 chain contains immunoreceptor tyrosine-based activation motifs (ITAMs), one each in γ, δ and ε and three in ζ. The features of these motifs are a pair of tyrosine residues separated by

9–11 amino acids. These tyrosines become rapidly phosphorylated by the Src-family kinase Lck following TCR stimulation; a required event for initiating TCR signalling [2]. The engagement of MHC-peptide by the TCR leads to clustering with CD4 or CD8 coreceptors as these coreceptors bind to invariant regions of the MHC molecule. Lck, a protein tyrosine kinase associated with the cytoplasmic tails of the coreceptors, is brought close to the cytoplasmic tails of the TCR complex and phosphorylates the ITAMs. The phosphorylated tyrosines in the ITAMs of the zeta chain provide docking sites to which a protein tyrosine kinase called ζ associated protein-70 (ZAP-70) attaches and becomes active. ZAP-70 then catalyzes the phosphorylation of a number of membrane-associated adaptor molecules which act as anchor points for the recruitment of several intracellular signal transduction pathways:

- **Phospholipase C-γ (PLC-γ)** is very crucial as it transduces TCR signals by hydrolyzing phosphatidylinositol bisphosphate (PIP2) to yield Diacyl glycerol (DAG), a membrane-associated lipid, and IP3, a diffusible second messenger that causes a rapid release of Ca^{2+} from the endoplasmic reticulum and opens Ca^{2+} channels in the cell membrane. DAG recruits a number of downstream proteins to the membrane, among them PKCθ and Ras GRP (RAS guanyl nucleotide-releasing protein), which is a guanine nucleotide exchange factor (GEF). RasGRP activates the small GTPase Ras, a crucial activator of Mitogen Activated Protein Kinase (MAPKs) signalling pathways in many cell types [3].

- **Calcium ion (Ca^{2+})** is involved in an unusually broad range of processes, including vision, muscle contraction, and many others. It is an essential element in many T-cell responses, including a pathway that leads to the movement of a major transcription factor, Nuclear factor of Activated cells (NFAT), from the cytoplasm into the nucleus. In the nucleus, NFAT supports the transcription of genes required for the expression of the T cell growth-promoting cytokines IL-2, IL-4 and others [4].

- **Protein kinase C (PKC)** is an enzyme, which affects many pathways, causes the release of an inhibitory molecule from the transcription factor NF-κB, allowing NF-κB to enter the nucleus, where it promotes the expression of genes required for T-cell activation. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is essential for a variety of T-cell responses and provides survival signals that protect T cells from apoptotic death [5].

- **The Ras/MAPKs (Mitoogen activated protein kinases) pathway** plays important role. Ras is a pivotal component of a signal-transduction pathway that is found in many cell types and is evolutionarily conserved across a spectrum of eukaryotes from yeasts to humans. Ras is a small G protein whose activation by GTP initiates a cascade of protein kinases known as the MAPKs pathway. As shown in phosphorylation of the end product of this cascade, MAPK (also called ERK), allows it to activate Elk, a transcription factor necessary for the expression of Fos. Phosphorylation of Fos by MAPKs allows it to associate with Jun to form AP-1, which is an essential transcription factor for T-cell activation [6].

MAPKs are evolutionarily conserved enzymes that are important in signal transduction. They play a diverse role in cell proliferation, cell death, cytokine production and cell differentiation. Three main families of MAPKs are found in mammalian cells: c-Jun-N-terminal kinases (JNK 1, 2 and 3); the extracellular signal-regulated kinases 1/2 (ERK1/2); and the p38 MAPK (p38 α, β, γ and δ) (Johnson., *et al*. 2002). They play diverse roles in the cell, ranging from apoptosis, cell differentiation, cell proliferation, stress response, to production of proinflammatory cytokines etc [7,8]. The p38 MAP kinase is also known as an important positive regulator of IFN-γ and Th1 differentiation. Persistent activation of p38 kinase resulted in increased IFN-γ production by Th1 effector cells [9]. The study of JNK1 and JNK2 knockout mice revealed the critical roles of JNK in CD4+ T cell proliferation and effector T cell function [10]. Both JNK and ERK are activated following T cell stimulation. Inhibition of the ERK pathway in transgenic mice does not significantly affect mature T cell proliferation [11]:

- **The Extracellular Signal-Regulated Kinases Signalling (ERK Signalling) pathway:** The ERK pathway is a hierarchical cascade originating at the cell membrane with receptors for mitogens or growth factors, which recruit via adapter proteins and exchange factors, the small guanosine triphosphatase (GTPase) Ras. Ras activates raf, a serine threonine kinase, which activates MEK (MAPK/ERK kinase). MEK in turn phosphorylates and activates ERK1 and ERK2, which translocate to the nucleus and transactivate transcription factors, changing gene expression to promote growth, differentiation or mitosis. By transducing signals through a cascade of kinases several options for control are introduced for amplifying and/or modifying the output signal [12].

- **The p38 Signalling Pathway:** p38 MAP-kinases is a stress activated kinase family all contain the same duel phosphorylation motif, thr-gly-tyr (TGY). The p38 MAPK family contains four members: p38α, p38β, p38γ and p38δ. Activation of p38 MAPK, an intracellular transduction pathway, results in the production of proinflammatory mediators. Activation of p38 results in its translocation into the nucleus and activation of a variety of transcription factors, including those essential for the production of proinflammatory mediators, such as chemokines and cytokines [13].

- **The SAPK/JNK Signalling pathway:** SAPKs are MAPKs shown to be activated by many different stress stimuli, hence their name stress-activated protein kinases (SAPKs) [14]. The same kinases were shown to phosphorylate c-Jun at Ser 64 and 73 hence the name Jun N-terminal kinases (JNKs). SAPKs/JNKs are activated by a variety of environmental stresses, inflammatory cytokines, growth factors and GPCR agonists. SAPKs bind and phosphorylate the transcription factor c Jun. c Jun is one component of the activator protein 1(AP-1) transcription factor complex. SAPK also phosphorylates the nuclear factor of activated T cells (NF-AT4), opposing its nuclear translocation during T cell activation. SAPKs translocate to the nucleus when activated, presumably to phosphorylate their nuclear targets [12].

The analysis of many signalling pathways has consistently revealed defective activation of the MAPKs ERK and JNK in response to anergic TCR –based signalling in several model systems [15]. It was noted that defective Ras signalling is the main functional defect in the anergic state. Feng., *et al*. [16] demonstrated that in LPS stimulated murine macrophages all three classes of MAP kinase, ERK1/2, JNK, and p38, are simultaneously activated, albeit with differential activation kinetics. However, their experiments using inhibitors selective for ERK1/2 (PD98059) and p38 (SB203580) show that while p38 plays an essential role in the induction of inducible NO synthase, ERK MAP kinases play only a minor role in promoting NO generation. In contrast, while p38 promotes induction of IL-12 (p40) mRNA, ERK activation suppresses LPS mediated IL-12 transcription.

Activation of transcription factors

Changes in gene expression represent the culmination of the TCR signalling pathway and are required for the T cell to gain full proliferative competence and the ability to produce effector cytokines. Three transcription factors in particular have been found to play a key role in TCR-stimulated changes in gene expression; these are NFκB, NFAT, and AP-1.

- **Nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)**: NFκB is the collective name given to the dimeric transcription factors of the Rel family. Activation of NFκB is primarily controlled via the nuclear cytoplasmic partitioning of NFκB. In the absence of an activating signal, NFκB is retained in the cytoplasm by tight binding to an inhibitory IκB protein. After activation an IκB kinase (IKK) complex, it phosphorylates IκB and targets it for ubiquitination and proteolysis via the 26S proteasome complexes. The degradation of IκB unmasks the nuclear localization sequence of NFκB, allowing translocation to the nucleus, where NFκB regulates the activity of its target genes [17]. A key step in NFκB activation is the activation of PKC and its translocation to lipid rafts. In anti CD3/28 stimulated T cells DAG and IP3 play important role in NFκB activation.

- **Nuclear Factor of Activated T cells (NFAT)**: In comparison with NFκB, the pathway leading to activation of NFAT is much simpler [18]. The rate-limiting step in NFAT activation is the removal of key phosphate groups from the N terminus of the NFAT protein. Phosphorylation of these residues masks the nuclear localization sequence on NFAT, and when the phosphates are removed NFAT can translocate to the nucleus and regulate the expression of various genes. The dephosphorylation of NFAT is specifically carried out by the Ca^{2+}, calmodulin-regulated phosphatase, calcineurin. Consequently, TCR-stimulated activation of PLCγ1, with the subsequent production of IP3 and increase in intracellular Ca^{2+}, is a critical component of NFAT activation.

- **Activator protein- 1 (AP-1):** Like NFκB activation, the activation of AP-1 requires PKCθ activation. It was observed that PKCθ/- mice fail to activate AP-1 in response to TCR stimulation [19]. The AP-1 transcription factor is composed of dimers of c-Jun and

c-Fos family proteins and can be activated both by phosphorylation of c-Jun by JNK and by up regulation of c-Fos and c-Jun expression [20]. AP-1 is also activated by PKCθ-independent pathways including the Ras/Raf/Mek/Erk pathway, which signals for increased expression of c-Fos. An important aspect of AP-1 function is its ability to form complexes with the NFAT and NFκB

Requirement of co-stimulatory signals for complete T-Cell activation

Optimal T-cell stimulation that leads to proliferation and other effector functions requires that a second, 'co-stimulatory' signal be delivered through a distinct cell surface receptor. Although several transmembrane proteins, including LFA1 and CD2, can provide co-stimulation in certain contexts, the archetypal costimulatory receptor is CD28. CD28 binds to B7-1 (also known as CD80) and B7-2 (also known as CD86), which are highly expressed by professional APCs such as dendritic cells. CD28 binds to B7-1 (also known as CD80) and B7-2 is highly expressed by professional APCs such as dendritic cells. Ligand binding of CD28 induces the phosphorylation of tyrosine-containing sequences in its cytoplasmic tail by Src-family kinases. This leads to the recruitment of several downstream proteins, including PI3K, Grb2, Vav and ITK. As each of these proteins is also recruited to the activated TCR complex, this suggests that CD28 stimulation does not activate qualitatively distinct pathways, but rather that it enhances TCR signalling quantitatively [22].

Clonal Anergy: Absence of Co-Stimulatory Signal

T cells recognition of an antigenic peptide–MHC complex sometimes results in a state of non- responsiveness called clonal anergy, marked by the inability of cells to proliferate in response to a peptide-MHC complex [23]. During a productive immune response, CD4+ T cells respond to effective signals by producing interleukin 2 (IL-2) and by proliferating. Effective signals require both ligation of TCRs with cognate antigens presented by class II MHC molecules on the surface of APCs and activation of co-stimulatory receptors, CD28, which recognize ligands expressed on the surface of APCs. When T cells receive stimulus only TCR signals in the absence of engagement of co-stimulatory receptors, they enter a state of anergy/unresponsiveness characterized by an inability to produce IL-2 or to proliferate upon re-stimulation. Such anergic T cells show a profound block in Ras/MAPK pathway that prevents activation of the AP-1 family of transcription factors (Fos/Jun) [24].

Altered cell signalling pathways in TB

TB pathogenesis is driven by a complex interplay between the host immune system and the survival strategies of the bacterium. The inflammatory response to *M. tb* infection is tightly regulated by both the host and the bacterium and protection against TB is based on cell-mediated immune responses. A consistent feature in TB patients has been the *in vitro* dysfunction of circulating T lymphocytes and mononuclear cells, especially at chronic stages of the disease [25]. T cells from chronically infected individuals show altered proliferative responses when they are primed *in vitro* with *M. tb* antigens and the phenomenon is called clonal anergy. Also, the *in vitro* activation and antigen presentation in both human and murine *M. tb* infected macrophages are known to be strongly inhibited [26,27]. Immunosuppression in TB is a complex and poorly understood phenomenon, which involves multiple mechanisms. Mycobacterial species are well adapted to the hostile environment of phagocytic cells and they use several strategies for survival within host cells that are not seen in other bacteria. A key event in the induction of a T-cell response is the repetitive stimulation of the T-cell receptor (TCR) by classical MHC class I, MHC class II or non-polymorphic MHC antigen-presenting molecules (e.g. CD1b, CD1c), leading to the activation of several tyrosine kinases which are responsible for eliciting T-cell responses, e.g. proliferation and cytokine secretion. Thus, variations in the expression of T-cell signal transduction molecules may be responsible for impaired immune function of T cells. Activation of MAPkinases, further downstream to calcium/PKC, is an important event for both cytokine production and cell activation. Our understanding of the mechanisms of interaction between mycobacteria and host cells, and of the consequent changes that are induced by mycobacteria in the host signalling machinery, is still incomplete. However, it is clear that some of the strategies that are used by mycobacteria for intracellular survival involve disruption of the host signalling machinery. The successful parasitization of macrophages by pathogenic mycobacteria involves the inhibition of several host-cell processes, which allows them to survive inside host cells. The host processes that are inhibited by pathogenic bacteria include the fusion of phagosomes with lysosomes, antigen presentation, apoptosis and the stimulation of bactericidal responses due to the activation of pathways involving MAPKs, IFN-γ and Ca^{2+} signalling.

Modulation of proximal TCR signalling

The proximal events following TCR engagement in conventional T cells include phosphorylation of tyrosine residues in ITAMs in the TCR-CD3 complex by the src kinase p56lck. ZAP-70 then binds to the phosphorylated CD3 complex, most notably to CD3ζ. These initial events initiate the activation of a signal transduction cascade, resulting in activation of PKC, mobilization of calcium, and activation of a Ras signalling cascade. Mahon., *et al.* [28] reported that inhibition of CD4+T-cell activation by *M. tuberculosis* glycolipids was seen with multiple subsets and was not mediated through TLR-2 or MyD88. *M. tb* glycolipids did not affect the downstream T-cell signalling pathways activated by PMA and ionomycin; however, they did suppress ZAP-70 phosphorylation, critical to proximal TCR signal transduction. In an another study Talreja., *et al*. [29] observed that PPD positive healthy controls produced significantly higher levels of IP3, intracellular Ca^{2+} and presented increased PKC activity when CD4+ T-cells were stimulated with *M. tb* H_{37}Rv cell lysate as compared to mantoux negative controls. Recently effect of *M. tb* antigens on TCR mediated signalling mechanisms leading IFN-γ secretion was observed and it was found that ESAT-6 decreased IFN-γ transcription and reduced expression of the transcription factors, ATF-2 and c-Jun, which normally bind to the IFN- γ proximal promoter and stimulate mRNA expression. ESAT-6 inhibited T cell IFN- γ secretion through mechanisms that did not involve cellular cytotoxicity or apoptosis. ESAT-6 bound to T cells and inhibited expression of early activation markers without reducing activation of ZAP70. It was concluded from that ESAT-6 directly inhibits human T cell responses to mycobacterial Ags by affecting TCR signalling pathways downstream of ZAP70 [30]. Reduced expression of CD3ζ chain in mycobacterial infections has been also reported [31].

Modulation of Ca^{+2} signalling

Calcium is a widespread intracellular second messenger that is known to play a critical role in the invasion of mammalian cells by a number of microorganisms. Several intracellular pathogens modify host cell calcium signalling during invasion. Examples include *Trypanosoma cruzi* induced modulation of fibroblast signalling [32], *Salmonella typhimurium* activates calcium dependent interleukin-8 release from endothelial cells [33] and *M. tuberculosis* inhibits macrophage calcium signalling leading to reduced phagosome-lysosome fusion and increased survival of pathogen in macrophage. Macrophages that are infected with killed or antibody- opsonized *M. tuberculosis* showed a sustained increase in cytosolic Ca^{2+} concentration compared with macrophages that are infected with live *M. tuberculosis* [34]. Furthermore, a reduced viability of *M. tb* was seen in macrophages that were treated with a Ca^{2+} ionophore, which artificially increases cytosolic Ca^{2+} concentration. In addition, macrophages that were infected with live *M. tb* showed a significant reduction in the amounts of Ca^{2+} bound calmodulin and phosphorylated CaMKII that were associated with the cytosolic face of the phagosomal membranes compared with phagosomes containing dead bacteria [35]. The delivery of lysosomal components to mycobacterial phagosomes can be blocked by using inhibitors of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) or by chelating cytosolic Ca^{2+}. These data indicate that pathogenic mycobacteria are able to suppress the increase in cytosolic Ca^{2+} that results from host cell infection, and thereby inhibit Ca^{2+} signalling pathways, which would otherwise lead to phagosomal maturation. It was reported that bacterial toxins interplay with T cell activation molecules like calcium ions mobilisation, PKC, IP3 turnover and MAP kinase molecules [36]. There are studies for *M. leprae* [37-40] and *M. tb* [29,41,42] where suppression of either of the three distinct biochemical pathways, viz. Ca-CN-NFAT pathway, PKC-NF-κB pathway and MAPK-AP-1 pathway by *M. leprae and M. tb* antigen(s) in anergized T cells of disease was observed. Few of the studies reported diminishing effect of mycobacterial on the activity of Ca^{2+}, IP3 turnover and PKC activation [34,40,43]. Decreased expression of CD3ζ and absence of the p65/p50 heterodimer of NF-κB was observed in patients with TB as compared with that of PPD-ve healthy controls [42]. Modulation of transcription factors (AP-1, NFκB) in anergised T cells have also been demonstrated [44]. Furthermore, effect of soluble antigens of *M. leprae* antigens have been demonstrated [39] to be effective in modulating the IL-2 gene expression, and thus disrupting TCR generated signal. Investigations of TCR and TCR/CD28-induced downstream signals in leprosy patients have been done and it was observed that *M. leprae* antigens curtail phosphorylation of both Erk1/2 and p38MAPK, consequently altering terminal signalling events like reduced binding of NFAT on IL-2 promoter and transcription of IL-2 gene [45]. If taken together, the above cited literature suggests that intracellular pathogens do modulate the immune responses by altering the signal transduction, and skews it for its own survival.

Alteration of MAPK signalling

The ability of the more virulent mycobacteria to limit the production of inflammatory mediators suggests that pathogenic mycobacteria initiate an active process for manipulating macrophage signal transduction pathways. The ability of pathogenic mycobacteria to limit MAPK activity has been suggested as an important virulence mechanism [46]. It was observed that mycobacterial secretory protein ESAT-6 could inhibit ERK1/2 activation in the nucleus of RAW264.7 cells. Feng., *et al.* reported that in LPS stimulated murine macrophages all three classes of MAPKs, ERK1/2, JNK, and p38, are simultaneously activated, albeit with differential activation kinetics. However, their experiments using inhibitors selective for ERK1/2 (PD98059) and p38 (SB203580) showed that while p38 plays an essential role in the induction of inducible NO synthase, ERK MAP kinases play only a minor role in promoting NO generation. In contrast, while p38 promotes induction of IL-12 (p40) mRNA, ERK activation suppresses LPS mediated IL-12 transcription [16]. The biochemical basis for the IL-2 production defect is a selective block in signal transduction to the MAPK and phosphorylation of ERK-1 and -2 is reduced, suggesting that the critical upstream molecule Ras is not activated [15,47]. Wang., *et al.* [30] observed that ESAT-6 inhibited both proliferation and IFN-γ production by purified CD3+T cells stimulated with anti-CD3 and anti-CD28, these finding indicated that these effects were independent of APCs. They also found that ESAT-6 inhibited expression of T cell activation markers and reduced expression of the transcription factors, ATF-2 and c-Jun, which normally bind to the IFN-γ proximal promoter and stimulate mRNA expression. Effect of *M. leprae* antigens on phosphorylation of MAPKinase was also studied and it was observed that WCL and MLSA significantly curtailed TCR-triggered phosphorylation of p38, similarly MLSA inhibited TCR-triggered Erk1/2 phosphorylation [45]. Joshi., *et al.* in 2006 [39] reported significantly curtailed the phosphorylation of ERK1/ERK2 by MLSA a *M. leprae* antigen in Jurkat T cells. *M. tuberculosis* has been reported to inhibit p38 activation, leading to inhibition of phagolysosomal fusion which helps in its intracellular survival [48]. *In vivo* studies have also established that *M. leprae* favours Erk1/2 phosphorylation for its survival and infection in Schwann cells [49]. We have previously reported that activation of ERK1/2 and p-38 was curtailed by *M. tuberculosis* antigens in TB patients whereas inhibition of only ERK1/2 not p-38 phosphorylation was observed in PPD+ve healthy individuals [50]. Pasquinelli., *et al.* [51] observed that IFN-γ production is regulated by ERK1/2 and p-38 MAPK signalling pathways in TB patients and it also involve CREB activation. Peng., *et al.* [52] reported that ESAT-6 induced inhibition of IFN-γ bγ activation of p-38 MAPK activity in T cells but did not affect activation of ERK1/2 or JNK. Feng., *et al.* in 1999 [16] observed the activation of ERK, JNK, and p38 MAPKs in J774 macrophages with differential kinetics after stimulation with LPS. The physiological relevance of such MAPKs regulation of macrophage effector function was demonstrated by their finding that synthetic *Leishmania lipophosphoglycan* exerts its inhibitory effects on the production of IL-12 by macrophages through stimulation of ERK MAPK which acts to suppress transcription of IL-12 (p40). Ganguly., *et al.* [53] observed that mycobacterial secretory protein ESAT-6 could inhibit ERK1/2 activation in the nucleus of RAW264.7 cells. Although activation of both p38and ERK1/2 is necessary for cytokine production but ERK1/2 specifically directly regulates both, the production of IL-2, cell cycle progression in activated T-cells and prevention of APC-induced anergy in T-cells [54], whereas p38 MAPK activation leads cell to a state of anergy [55] and suppressor function due to reduced cell cycle progression [56]. Nuclear transcription factors such as NF-κB and NFAT appear to play a central role in both T cell activation and the regulation of certain cytokine genes. Changes in gene expression represent the culmination of the TCR signaling pathway and defective proliferative competence and the ability to produce effector cytokines. NF-κB is a nuclear transcription factor ubiquitously involved in gene expression for a variety of inflammatory mediators including IL-2, IL-6, IL-8, TNF-α and IFN-γ etc. NFAT, a key transcription factor for several molecules [48]. It plays a crucial role in transcriptional activation of cytokine gene expression of IL-2, TNF-α and GM-CSF etc. NFAT, a key transcription factor for several cytokines including IL-2, translocates from cytosol to nucleus following stimulation with TCR ligands, calcium ionophores, or other agents that induce an increase in cytoplasmic free calcium. A key step in NFκB activation is the activation of PKC and its translocation to lipid rafts. In CD3/28 stimulated T cells DAG and IP3 plays important role in NFκB activation. When cytosolic calcium levels are low, phosphorylation of glycogen synthase kinase 3 (GSK-3) induces nuclear export of NFAT [3] and could lead to anergy. NFAT in combination with AP-1 regulates IL-2 gene transcription and NAFT1 regulates a majority of genes implicated in anergy [57]. Thus, inhibition of NFAT activity by mycobacterial antigens could not only trigger cellular anergy programme in T-cells but could also inhibit IL-2 gene transcription, a hallmark for T-cell activation for mounting an effective CMI against intracellular pathogens. Defective PKC activation, calcium mobilization, NFAT, NFκB, Ras-ERK-AP-1

activation and reduced IL-2 production have been found in regulatory T-cells [58], therefore it can be postulated that *M. tb* induces T-cells to maintain hyporesponsive state by suppressing the induction and propagation of TCR-initiated signals to control IL-2 production and cell proliferation. Zea., *et al.* [42] observed that patients with active pulmonary TB have a significant decrease in the expression of CD3ζ, which is an important signal-transduction protein in T cell activation, and lack the p65/p50 NF-κB heterodimer, which is important in the activation of certain T cell genes. Their data suggest that these alterations could in part explain the loss of a protective immune response against *M. tb,* and they may help to understand the mechanisms that lead to T cell dysfunction in TB. Zea., *et al.* in 1998 [37] also observed changes in the expression of CD3ζ and p56lck and the absence of NF-κB p65 in the nucleus in patients with leprosy. Dagur., *et al.* in 2010 [45] reported reduced binding of NFAT on IL-2 promoter in leprosy patients. Tchou- Wong., *et al.* [59] revealed specific binding of nuclear protein to the NFκB site upon induction with *M. tb*. Altogether, results suggest that *M. tb* interferes with TCR/CD28- induced upstream as well as downstream signalling events resulting in reduced cytokine production and thus an inhibition in T-cell proliferation, which might be responsible for T-cell unresponsiveness leading to stage of immunosuppression and consequently, for the progression of disease [60].

Conclusion

Mycobacterial species are well adapted to the hostile environment of phagocytic cells, and they use several strategies for survival within host cells that are not seen in other bacteria. Our understanding of the mechanisms of interaction between mycobacteria and host cells, and of the consequent changes that are induced by mycobacteria in the host signalling machinery, is still incomplete. There is a need to collect more information for T cell activation in TB patients and to find out the role of secretory antigens of *M. tuberculosis* in modulation of the immune responses by altering the signal transduction pathways. The study of T cell signalling pathways evoked in response to secretory mycobacterial antigens and their role in inducing anergy may offer a practical and efficient strategy to design a better subunit vaccine against TB. There is an urgent need for more effective controls of TB worldwide, chemotherapeutic regimens are effective against drug-susceptible TB but they are for long term and thus leading to patient non-compliance. The real progress will require more detailed knowledge of the host immune responses. However, it is clear that some of the strategies that are used by mycobacteria for intracellular survival involve disruption of the host signalling machinery. These observations of molecular and functional characteristics in TB may provide new tools to study and monitor patients, to determine how these characteristics effect the development of immune dysfunction, and to study new pathways to block suppressor mechanisms. This endeavour would re-establish that the function of the immune system combined with anti-TB therapy will benefit the clinical outcome in patients with TB.

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